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Synergistic Effect of Seaweed Extract and Urea Fertilizer on Growth and Biochemical Parameters of *Triticum aestivum* at Vegetative Stage

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Abstract

This study was designed in order to investigate the Synergistic effect of seaweed extract (*Nizamuddinia zanardinii*) and urea fertilizer on growth and biochemical parameters of *Triticum aestivum* at vegetative stage. Experiment was factorial with completely randomized design, with three replications at five levels (0, 5, 10, 15 and 20 percent) of brown seaweed extract and three levels of urea (0, 25 and 50 percent of recommended amount). According to the results, maximum dry weight of leaf, shoot and root (0/85, 0/87 and 0/09 g), shoot and root length (46/67 and 13/5 cm), content of total chlorophyll and carotenoid (0/73 and 0/16 mgg-1fr.wt), activity of peroxidase and ascorbateperoxidase enzyme (8/6 and 0/82 ΔOD.min^{-1}/mg.protein) was observed at 10% concentration of brown seaweed extract with 25% recommended rate of urea fertilizer. The maximum amount of malondialdehyde (19/42 mM/g.FW) and Catalase activity (0/64 ΔOD.min^{-1}/mg.protein) was observed in 20% concentration of brown seaweed.

Keywords: Ascorbateperoxidase, Catalase, malondialdehyde, Peroxidase, Photosynthetic pigments

Introduction

In the vast marine ecosystem, several forms of life starting from unicellular to multicellular flourish, multiply and disintegrate. It is believed that the first living cell that appeared on the planet earth emerged from the ocean. In all its form, the life has developed from the growth of unicellular algae. About 90% of the species of marine plants are algae and about 50% of the global photosynthesis is derived by algae only. Marine plants comprise of algae, sea grasses, mangroves and sand dune vegetation. The algae are of different shapes and sizes. The microscopic algae are known as phytoplankton and macroscopic ones as seaweeds (Paul & Yuvaraj, 2014). Seaweeds are one of the most important marine resources of the world and being used as human food, animal feed and raw material for many industries. They are also used as manure for agricultural and horticultural crops, due to the presence of minerals, trace elements and plant growth regulators which occur in water soluble form (Christobel, 2008).

Seaweed meals provide an approximately equivalent amount of N, less P but more K, total salts and readily available micronutrients compared to most animal manures (Vijayanand *et al*., 2014). Applications of chemical fertilizers certainly compensate the deficiency of nutrients in soil. Whereas, in excess it affects soil and plants in due course. Chemical fertilizers have degraded the fertility of the soil by making it acidic, rendering it unsuitable for raising crops (Sridhar & Rengasamy, 2010). Unlike chemical fertilizer, fertilizers derived from seaweeds (*Fucus*, *Laminaria*, *Ascophyllum*, *Sargassum* etc.) are biodegradable, non-toxic, non-polluting and nonhazardous to human, animals and birds (Paul & Yuvaraj, 2014). Farmers, all throughout the world are switching over to organic fertilizers. Seaweed manure besides increasing the soil fertility increases the moisture holding capacity and supplies adequate trace metals thereby improving the soil structure (Sridhar & Rengasamy, 2010; Paul & Yuvaraj, 2014).

Numerous studies have revealed a wide range of beneficial effects of seaweed foliar spray applications on plants in terms of health of plants, increase in rate of growth and antioxidant properties, resistance to frost, fungal and insects attach, higher yield of 25 to 30% etc (Kalaivanan et al., 2012).

Aqueous extract of *Rosenvingea intricata* when applied as a foliar spray on *Cyamopsis tetragonaloba* showed an increased yield and quality of fruit (Thirumaran *et al*., 2009).

1% of seaweed liquid fertilizer of *Sargassum wightii* plus mixture 50% recommended rate of urea fertilizers promoted the growth parameters and biochemical constituents such as shoot and root height, shoot and root dry weight, photosynthetic pigments, protein and Carbohydrate (Sridhar & Rengasamy, 2010).

Considering the above facts, the main objective of this paper is to evaluate the synergistic effect of seaweed extract (*Nizamuddinia zanardinii*) and urea fertilizer on growth and biochemical parameters of wheat at vegetative stage.

Materials and methods

Collection of seaweeds

The seaweed used in the present study were *Nizamuddinia zanardinii* belonging to the classes Phaeophyceae. They were collected from the coastal area of Chabahar, Iran (25° 17_ N and 60° 37_ E) during November, 2013. The seaweed were handpicked and washed thoroughly with seawater to remove all the unwanted impurities.

Preparation of seaweed liquid fertilizer

Freshly collected seaweeds were shade dried for ten days. Dried material was finely powdered. Fifty gram of finely powdered material was extracted with 500 mL boiling water for 60 min. The hot extract was filtered through a double layered cheese cloth and allowed to cool at room temperature. The resulting extract was taken as 100% concentration of the SLF (Ramarajan *et al*., 2012; Sivasankari *et al*., 2006). As the seaweed liquid fertilizers contained organic matter, the seaweed liquid fertilizers were refrigerated between 0 and 4 °C.

Experimental design and treatments

The trial was carried out during 2014-2015 years in the Shavoor on the Experimental field of Institute of Agricultural Sciences of Khozestan, Ahvaz. The experimental site is located at 31° 50′ N and 48° 28′ E. Experiment was factorial with completely randomized design, with three replication at five levels (0, 5, 10, 15 and 20%) of brown seaweed extract and three levels 0, 25 and 50 percent of recommended amount of 46 percent urea fertilizer (the recommended amount of urea fertilizer was 300 kg per hectare). The seeds of wheat (*Triticum aestivum* cv. Chamran 2) were collected from Chamran University, Khozestan, Ahvaz, Iran. They were surface sterilized with 5% sodium hypochlorite. The seeds with uniform size, color and weight were chosen for the experimental purpose. Plots included 30 cm diameter pots with a capacity of 20 kg. Since 150 Kg of seed used per hectare and our pots had about 0/07 m^2 area, approximately one gram of seed per pot was given. Recommended amount of urea fertilizer was mixed with soil. Different concentrations of seaweed extract were applied as foliar spray at 30 days after planting. The plants were harvested at vegetative stage (60 days old).

Physico-chemical analyses of seaweed extract and soil

The colour, pH, nitrate, phosphorus, potassium, iron, zinc, copper and manganese content of seaweed extract were analyzed and are described in table 1.

The pH, electrical conductivity, nitrate, phosphorus, potassium, copper, zinc, manganese, iron and soil texture content of pots soil from a depth of 0-30 cm were analyzed and are described in table 2.

Growth and biochemical analysis

The growth parameters including shoot and root length, shoot, root and leaf dry weight were calculated. The biochemical constituents such as total chlorophyll and carotenoid (Lichtenthaler, 1987), malondialdehyde content (Davey *et al.*, 2005) were estimated in Wheat. Also changes in activity of antioxidant enzymes such as peroxidase (Hoyle, 1972), catalase (Aebi, 1984) and ascorbate peroxidase (Chen & Asada, 1989) were studied.

Results

The physico-chemical properties of seaweed extract of *Nizamuddinia zanardinii* were analyzed and are presented in Table 1. The colour of the seaweed extract was brown. The pH of seaweed extract was 7.2. The content of Iron was higher than other elements and the content of Phosphorus was less than others.

Table 1. Physico-chemical properties of *Nizamuddinia zanardinii*

Manganese (ppm)	Copper (ppm)	Zinc (ppm)	Iron (ppm)	Potassium (ppm)	Phosphorus (ppm)	Nitrate (ppm)	pH	Color
10	4	6	95	62.3	3.2	12.86	7.2	Brown

The physico-chemical properties of soil in the pots were analyzed and are presented in Table 2.

Table 2. Physico-chemical properties of soil

soil texture	Micro-absorbable elements (ppm)				K(ppm)	P(ppm)	(%)N	Ec	pH	Soil depth (cm)
	Fe	Mn	Zn	Cu						
Clay-loam	15/7	3/2	2/8	2/8	360	10	0/08	3/1	7/2	0-30

Table 3. Analysis of variance for growth parameters of wheat at vegetative stage

Source	Degrees of Freedom			Mean Square		
		Dry weight of leaf	Dry weight of shoot	Dry weight of root	Shoot length	Root length
Concentration of Seaweed extract (A)	4	0/310**	0/333**	0/004**	105/419**	66/314**
Urea (B)	2	0/197**	0/278**	0/003**	66/843**	49/041**
Interact A and B	8	0/0143**	0/04**	0/001**	4/415**	4/219**
Error	30	0/002	0/001	0/002	0/642	0/394
CV (%)		9/19	6/48	15/20	1/98	7/52

** denotes significant at 1% level

The effect of seaweed extract of *Nizamuddinia zanardinii* on growth parameters of wheat at vegetative stage is presented in Table 3 and figures 1-5.

The highest values of dry weight leaf, shoot and root (0/85, 0/87 and 0/09 g), shoot and root length (46/67 and 13/50 cm) were found at combination 10% concentration of seaweed extract with 25% recommended rate of chemical fertilizers and the lowest values of dry weight leaf, shoot and root (0/32, 0/28 and 0/03 g), shoot and root length (37/17 and 5/17 cm) were observed at 20% concentration of seaweed extract. The result of the present study indicated that the treatments which were laid using the mixture of seaweed extract along with 50% of chemical fertilizer, had significant decrease in studied growth parameters in comparison to those treatments which were mixture of seaweed fertilizer along with 25% recommended rate of urea fertilizer.

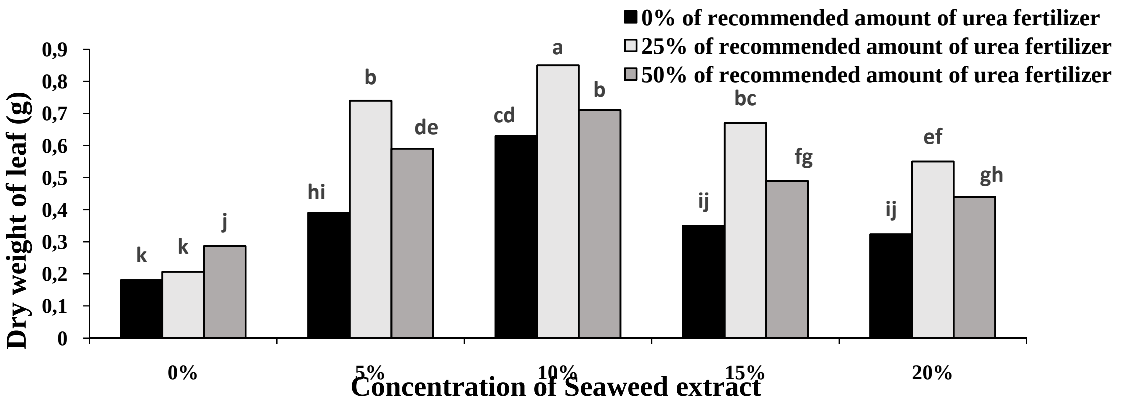

Figure 1. Interaction seaweed extract and urea on the leaf dry weight of wheat at vegetative stage. Different letters in a single column show statistically significant differences according to Duncan's multiple range test (p = 0.05)

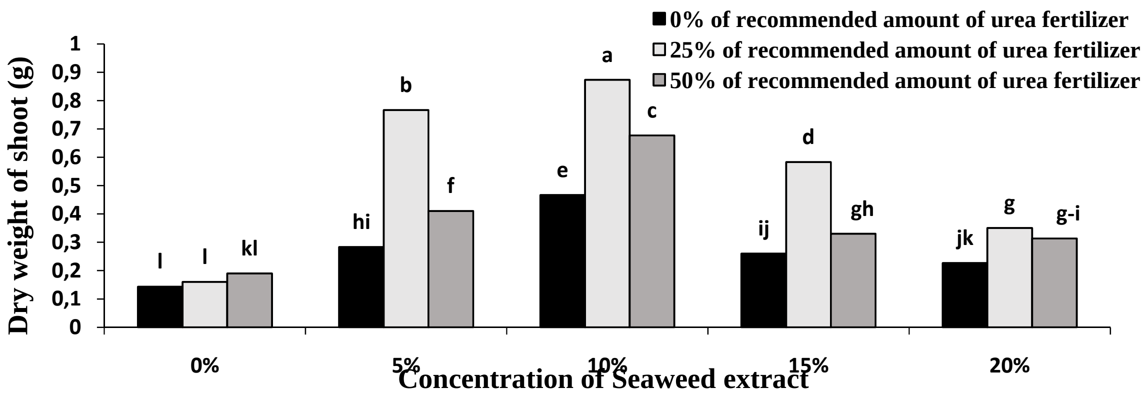

Figure 2. Interaction seaweed extract and urea on the shoot dry weight of wheat at vegetative stage. Different letters in a single column show statistically significant differences according to Duncan's multiple range test (p = 0.05)

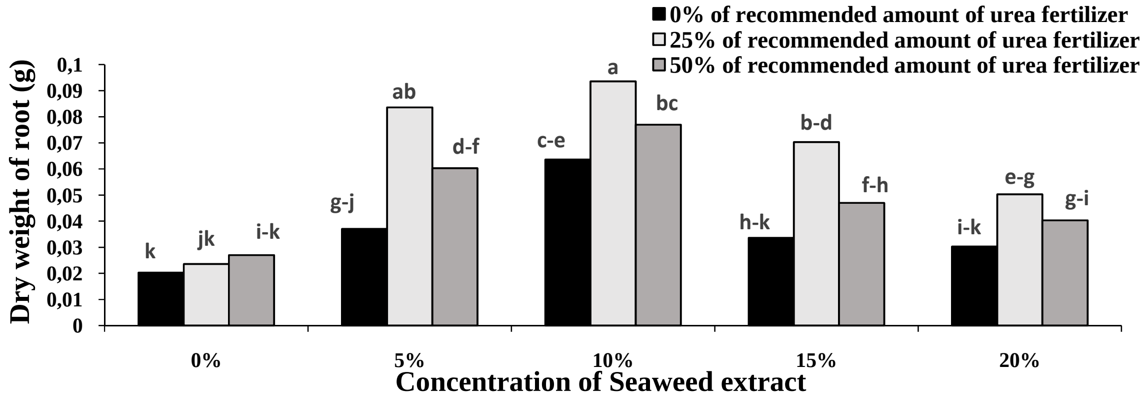

Figure 3. Interaction seaweed extract and urea on the root dry weight of wheat at vegetative stage. Different letters in a single column show statistically significant differences according to Duncan's multiple range test (p = 0.05)

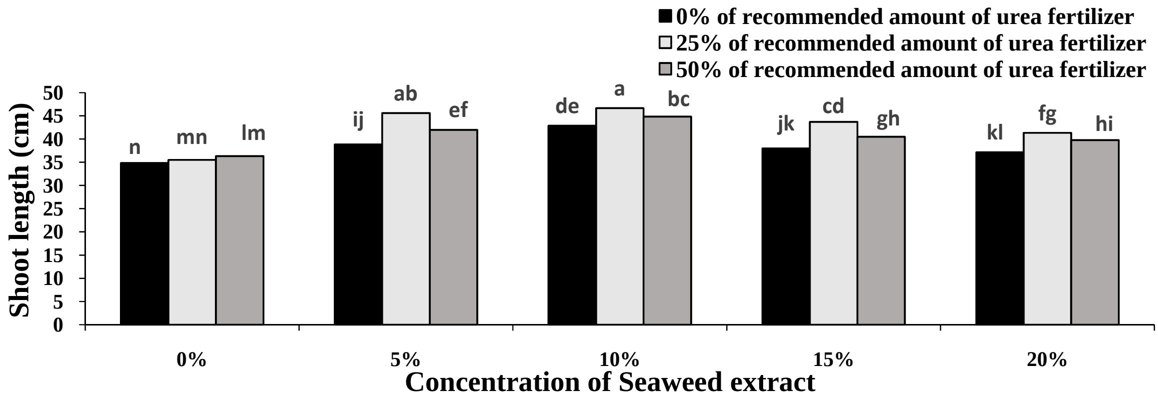

Figure 4. Interaction seaweed extract and urea on the shoot length of wheat at vegetative stage. Different letters in a single column show statistically significant differences according to Duncan's multiple range test (p = 0.05)

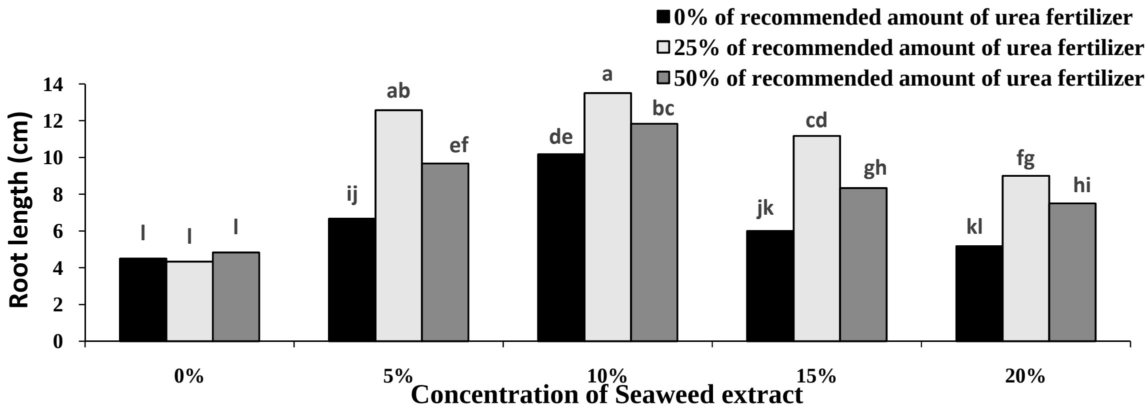

Figure 5. Interaction seaweed extract and urea on the root length of wheat at vegetative stage. Different letters in a single column show statistically significant differences according to Duncan's multiple range test (p = 0.05)

The effect of seaweed extract of *Nizamuddinia zanardinii* on biochemical parameters of wheat at vegetative stage are presented in Table 4 and figures 6-11.

Table 4. Analysis of variance for biochemical parameters of wheat at vegetative stage

Source	Degrees of Freedom	Mean Square					
		Total chlorophyll	Carotenoid	Peroxidase	Ascorbate peroxidase	Catalase	Malondialdehyde
Concentration of Seaweed extract (A)	4	0/056**	0/002**	14/807**	0/232**	0/111 **	23/296**
Urea (B)	2	0/022**	0/004**	8/752**	0/142**	0/069 **	17/424**
Interact A and B	8	0/019**	0/001**	0/620**	0/007**	0/008 **	3/886**
Error	30	0/001	0/001	0/002	0/002	0/001	0/002
CV (%)		1/10	1/64	0/63	7/95	1/41	0/26

** denotes significant at 1% level

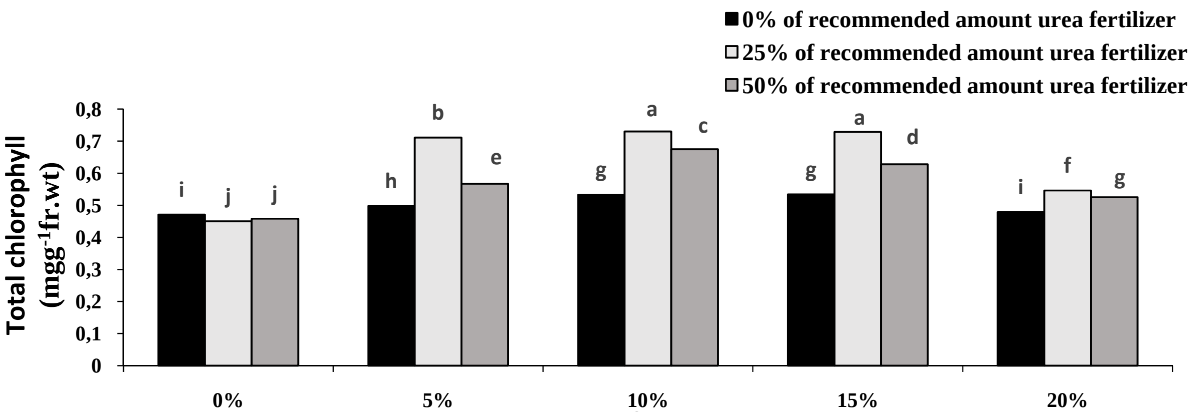

Figure 6. Interaction seaweed extract and urea on the total chlorophyll of wheat at vegetative stage. Different letters in a single column show statistically significant differences according to Duncan's multiple range test (p = 0.05)

The highest values of total chlorophyll and carotenoid (0/73 and 0/16 mgg-1fr.wt), peroxidase and ascorbate peroxidase activity (8/60 and 0/82 ΔOD.min^{-1}/mg.protein) were found at combination 10% concentration of seaweed extract with 25% recommended rate of chemical fertilizers and the lowest values of total chlorophyll and carotenoid (0/48 and 0/1 mgg-1fr.wt), peroxidase and ascorbate peroxidase activity (5/12 and 0/45 ΔOD.min^{-1}/mg.protein) were observed at 20% concentration of seaweed extract. The result of the present study indicated that the treatments which were laid using the mixture of seaweed extract along with 50% of chemical fertilizer, had significant decrease in total chlorophyll and carotenoid content, amount of peroxidase and ascorbateperoxidase activity in comparison to those treatments which were mixture of seaweed fertilizer along with 25% recommended rate of urea fertilizer.

The highest values of Catalase activity (0/64 ΔOD.min^{-1}/mg.protein) and malondialdehyde (19/42 mM/g.FW) were found at 20% concentration of seaweed extract and the lowest values of Catalase activity (0/31 ΔOD.min^{-1}/mg.protein) and malondialdehyde (14/21 mM/g.FW) were observed at combination 10% concentration of seaweed extract with 25% recommended rate of chemical fertilizers.

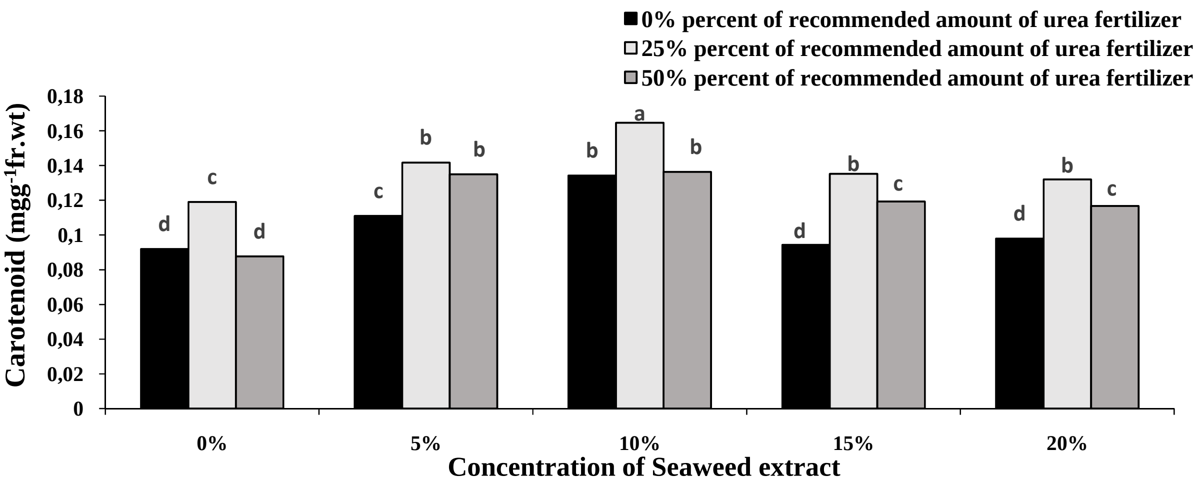

Figure 7. Interaction seaweed extract and urea on the carotenoid of wheat at vegetative stage. Different letters in a single column show statistically significant differences according to Duncan's multiple range test (p = 0.05)

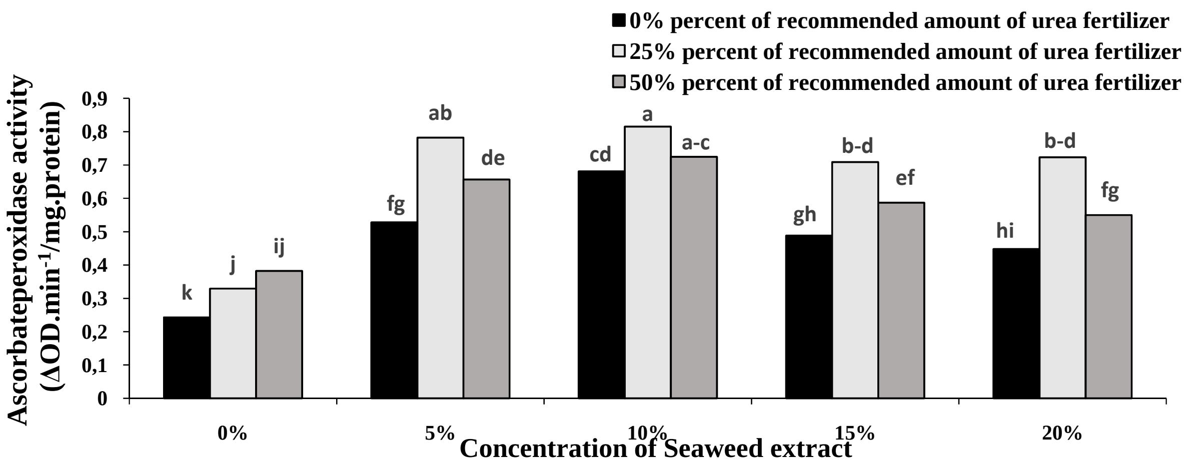

Figure 8. Interaction seaweed extract and urea on the ascorbate peroxidase activity of wheat at vegetative stage. Different letters in a single column show statistically significant differences according to Duncan's multiple range test (p = 0.05)

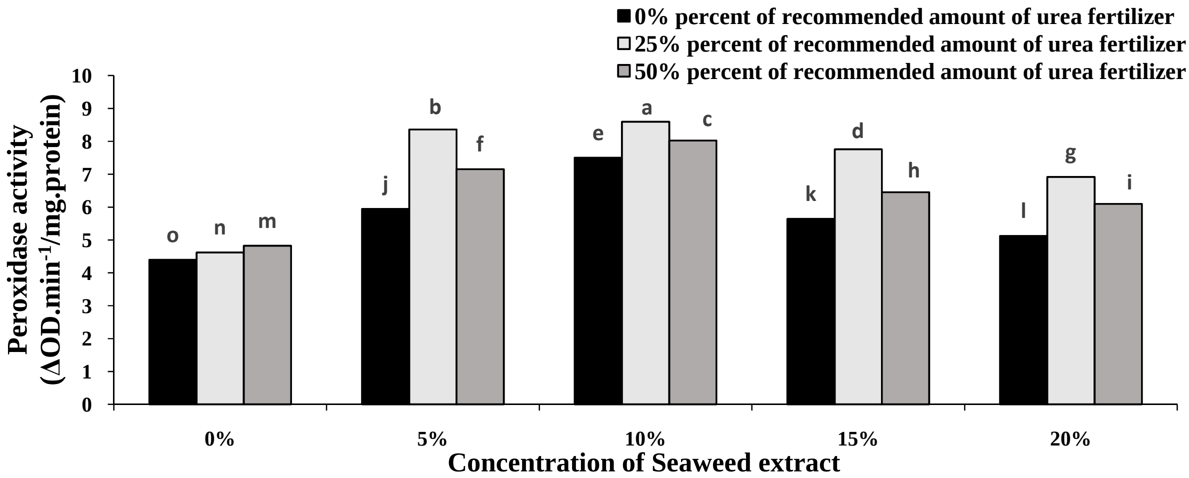

Figure 9. Interaction seaweed extract and urea on the peroxidase activity of wheat at vegetative stage. Different letters in a single column show statistically significant differences according to Duncan's multiple range test (p = 0.05)

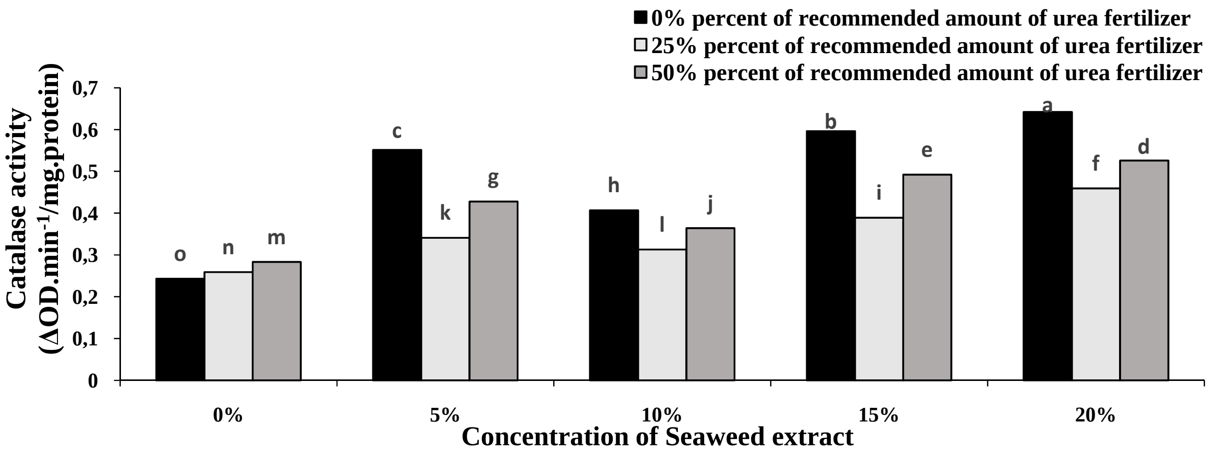

Figure 10. Interaction seaweed extract and urea on the catalase activity of wheat at vegetative stage. Different letters in a single column show statistically significant differences according to Duncan's multiple range test (p = 0.05)

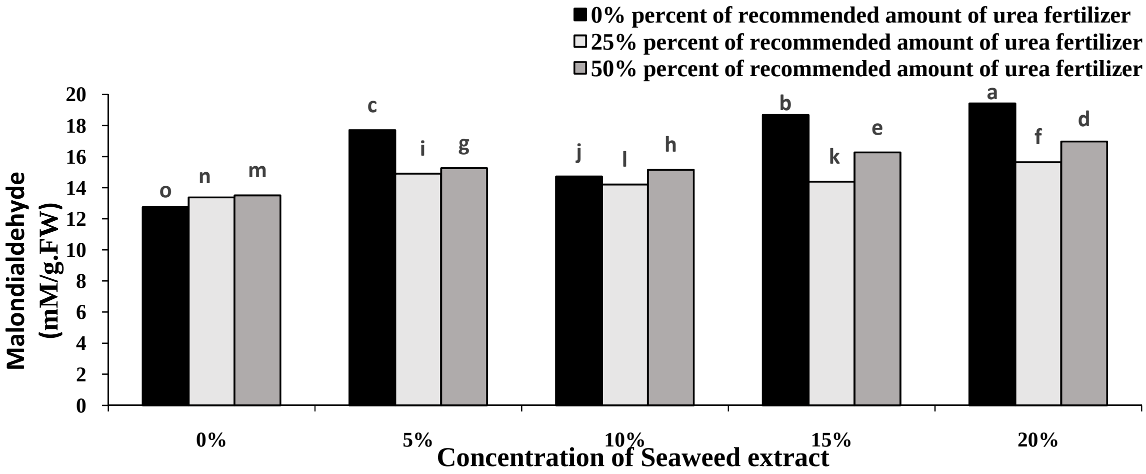

Figure 11. Interaction seaweed extract and urea on the malondialdehyde content of wheat at vegetative stage. Different letters in a single column show statistically significant differences according to Duncan's multiple range test (p = 0.05)

Discussion

Organic farming is one of the fastest growing sectors of agriculture worldwide. Its main objective is to create a balance between the interreconnected systems of soil organisms, plants, animals and human. The target in the application of organic fertilizers should therefore two fold- first to obtain reasonable yields and second to increase soil fertility, water holding capacity to optimum levels. In modern agriculture, special emphasis is placed on soil amendment, improved agricultural equipments, techniques and improved varieties of tolerant plants for increasing the quality and quantity of yield per hectare (Badar et al., 2015). In the developing world, the use of seaweed liquid fertilizer should be urged to avoid environmental pollution by heavy doses of chemical fertilizer in the soil (Narasimha Rao & Chatterjee, 2014).

The results of the present study showed, using of seaweed extract led to a significant increase in the studied growth parameters in comparison to that of the control. The combination of seaweed extract with 25% recommended rate of urea fertilizer caused to significant increase in mentioned parameters, which indicated the synergistic influence of the seaweeds and urea chemical fertilizer.

Similar results were also observed in *Arachis hypogea* L. (Selvam & Sivakumar, 2014; Kannathasan *et al.*, 2008), *Sesamum indicum* (Gandhiyappan & Perumal, 2001) and *Vigna mungo* (Kalaivanan *et al*., 2012).

The growth enhancing potential of seaweeds might be attributed to the presence of carbohydrates, phenylacetic acid, macro and micro elements, vitamins and plant growth regulators like auxin, cytokinin and gibberellins (Jothinayagi & Anbazhagan, 2009; Christobel, 2008).

Auxin plays a key role in growth of plants. By increasing and activating H-ATPase pumps, Auxin sends protons to apoplast space. Resulting from decreasing pH, some enzymes will be activated which loosen cell wall. Auxin also by increasing production of some soluble materials inside the cell, decrease the water potential and finally let water enter to the cells. These procedures lead to growth of cell and finally growth of plant (Taiz & Zeiger, 2010). These cytokines are active at very low concentrations and regulate a number of plant functions including cell division, protein, enzyme formation, leaf aging and senescence, shoot elongation, and fruit set. Gibberellic acid is one of most important growth stimulating substance used for promoting cell elongation, cell division and thus to promote growth and development of many plant species (Kasim *et al*., 2015).

Enhanced chlorophyll and carotenoid contents at lower concentration of SLF have been reported for *Triticum aestivum* (Kumar & Sahoo, 2011) and *Cyamopsis tetragonoloba* (Sivasankari Ramya *et al.*, 2011).

The increase in the photosynthetic pigment contents were probably due to the presence of minerals such as Fe, Ni, Cu and Mg in the seaweeds. Iron, copper, and magnesium are essential elements which act as catalysts for the synthesis and efficient function of the chlorophyll. The plant growth regulators from the seaweed may also have been responsible for the elevated synthesis of plant pigments (Elumalai & Rengasamy, 2012).

According to the results of present study, using of 10% concentration of seaweed extract with 25% recommended rate of urea fertilizers led to a increase in peroxidase, ascorbate peroxidase activity and a decrease catalase activity and malondialdehyde content. These results coincide with the studies on *PhaSseolus aureus L*. (Christobel, 2008), *Cyamopsis tetragonoloba* L. (Thambiraj, *et al.*, 2012) and *Triticum durum* L. (Chernane *et al.*, 2015).

Membrane damagemight be a result of initiated oxygen stress and the accumulation of reactive oxygen species leading to disturbances in membrane configuration, andoxidation of cell membrane fatty acids. Such damage can result from various mechanisms including oxidation and crosslinkage of protein thiols, inhibition of key membrane proteins as H+-ATPase, and changes to the composition and fluidity of membrane lipids (Kasim *et al*., 2015).

Plants possess several protective mechanisms to cope with ROS in which enzymes as well as reducing metabolites. The enzymatic antioxidant system can be divided in two categories. One reacts with ROS and keeps them at low levels (peroxidase and catalase), and the other regenerates the oxidised antioxidants (ascorbate peroxidase). The reduction of H2O2 by ascorbate can occur directly or it can be catalysed by ascorbate peroxidase. Then, the oxidised from of ascorbate can be reduced enzymatically by deshydroascorbate reductase using glutathione as a substrate, which in turn is reduced by glutathione reductase in the presence of NADPH. Catalase (CAT) is a tetramerichromoprotein that exists as multiple isoenzymes encoded by nuclear genes. Ascorbate peroxidase is the most important peroxidase in H2O2 detoxification (Chernane *et al.,* 2015). Normally the higher activity of the peroxidase appears to be the results of greater mobilization of storage compound and energy sources (Christobel, 2008).

Conclusion

The present study is an important step towards the utilization of the extracts of the seaweeds *Nizamuddinia zanardinii* to improve the growth of wheat. The presence of nitrogen, magnesium, potassium and some trace elements in seaweeds make an excellent choice as organic fertilizers. The practice of application of ecofriendly seaweed liquid extracts to pulses is recommended to the growers for attaining better yield.

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